Application Number 09/831,496 Attorney Docket Number 0425-0837P Page 2 of 13

just before a separation column in a high performance liquid chromatographic apparatus having a flow velocity gradient of 250 microliters per minute or less, wherein said diffusion promoting device comprises a solvent inlet tube and a solvent outlet tube and wherein said device includes at least one feature selected from the group consisting of (i) having a solvent inlet tube and a solvent outlet tube with different inner diameters, (ii) having a solvent outlet tube connected to a solvent inlet tube at an acute angle, a right angle, or an obtuse angle, and (iii) having a solvent outlet tube connected to a solvent inlet tube' by a connecting part having a diameter that is larger than the diameters of the diameters of the solvent inlet and outlet tubes.

- 8. (twice amended) A high performance liquid chromatographic apparatus having a flow velocity gradient of 250 microliters per minute or less, said apparatus comprising a diffusion promoting device that comprises a solvent inlet tube and a solvent outlet tube, wherein said device includes at least one feature selected from the group consisting of (i) having a solvent inlet tube and a solvent outlet tube with different inner diameters, (ii) having a solvent outlet tube connected to a solvent inlet tube at an acute angle, a right angle, or an obtuse angle, and (iii) having a solvent outlet tube connected to a solvent inlet tube by a connecting part having a diameter that is larger than the diameters of the diameters of the solvent inlet and outlet tubes, said diffusion promoting device being positioned just before the separation column.
- 9. (twice amended) The high performance liquid chromatographic apparatus according to Claim 8, in which the diffusion promoting device is connected between a component concentration column and the separation column.



10. (thrice amended) A high performance liquid ehromatographic apparatus having a flow velocity gradient of 250 microliters per minute or less, said apparatus in which a solvent pump (P1), an injector (I), and a switching valve (V) are connected in this order in one line; and a solvent pump (P2), a switching valve (V), a diffusion promoting device (DU) that comprises a solvent inlet tube and a solvent outlet tube, wherein said device includes at least one feature selected from the group consisting of (i) having a solvent inlet tube and a solvent outlet tube with different inner diameters, (ii) having a solvent outlet tube connected to a solvent inlet tube at an acute angle, a right angle, or an obtuse angle, and (iii) having a solvent outlet tube connected to a solvent inlet tube by a connecting part having a diameter that is larger than the diameters of the diameters of the solvent inlet and outlet tubes, a separation column (C), and a detector (D) are connected in another line.



11. (thrice amended) A high performance liquid chromatographic apparatus having a flow velocity gradient of 250 microliters per minute or less, said apparatus in which a solvent pump (P1), a switching valve (V), a solvent mixer (MC), and a switching valve (V) are connected in this order in one line; a solvent pump (P2), a switching valve (V), a diffusion promoting device (DU) that comprises a solvent inlet tube and a solvent outlet tube, wherein said device includes at least one feature selected from the group consisting of (i) having a solvent inlet tube and a solvent outlet tube with different inner diameters, (ii) having a solvent outlet tube connected to a solvent inlet tube at an acute angle, a right angle, or an obtuse angle, and (iii) having a solvent outlet tube connected to a solvent inlet and outlet tube connected to a solvent inlet and outlet tubes, a separation column (C), and a detector (D) are connected in another line; and



a switching valve (V), a component concentration column (M), and a switching valve (V) are connected in a different line.

12. (twice amended) A method for analyzing a trace amount of a component in a sample with improved detection sensitivity for use in the high performance liquid chromatographic apparatus according to Claim 10, which comprises trapping the target component in the component concentration column (M) by means of a mobile phase discharged from the solvent pump (P1); discharging a different mobile phase from the solvent pump (P2) by turning the switching valve; and eluting the target component from the separation column (C) through diffusion of the target component using the diffusion promoting device (DU).



13. (twice amended) A method for analyzing a trace amount of a component in a sample with improved detection sensitivity for use in the high performance liquid chromatographic apparatus according to Claim 11, which comprises injecting the target component into the component concentration column (M) while filling a solvent in the solvent mixer (MC) by means of the solvent pump (P1); discharging a mobile phase from the pump (P2) by turning the switching valve; and eluting the target component from the separation column (C) through diffusion of the target component using the diffusion promoting device (DU).

Add new claims 15-18:



15. (new) The high performance liquid chromatographic apparatus according to one of Claims 8, 10, and 11, wherein said diffusion promoting

device (ii) has a solvent outlet tube connected to a solvent inlet tube at an acute angle, a right angle, or an obtuse angle.

16. (new) The high performance liquid chromatographic apparatus according to one of Claims 8, 10, and 11, wherein a frit is inserted into at least one of the solvent inlet tube, the solvent outlet tube, and a position between the solvent inlet tube and the solvent outlet tube.



- 17. (new) The high performance liquid chromatographic apparatus according to Claim 16, wherein the frit is a sintered filter, a ceramic, a metal mesh, or a cellulose fiber.
- 18. (new) The high performance liquid chromatographic apparatus according to one of Claims 8, 10, and 11, wherein the low flow velocity gradient high performance liquid chromatographic apparatus is a gradient micro high performance liquid chromatographic apparatus, a gradient semimicro high performance liquid chromatographic apparatus, or a gradient capillary high performance liquid chromatographic apparatus.

Attached hereto is a marked up version showing the claim amendments made by this Amendment.